APPLICANTS: Rush *et al.*
U.S.S.N.: 10/777,893

REMARKS

Applicants note that the Examiner has withdrawn the previous rejections under 35 U.S.C. §112, 1st paragraph (written description). The Examiner has also withdrawn the prior allowance of claims 30-33 and 36-39; as well as the prior statement that the claimed subject matter is free and clear of all prior arts. Some of the newly made rejections are based, in part, on the same art previously cited in the first Office Action in this case. The Examiner has stated that claim 53 is free and clear of all prior art.

The present response is filed subsequent to a second in-person interview with the Examiner, conducted on August 15, 2006, in which the outstanding claim rejections were discussed, the newly cited art distinguished, and certain claim amendments presented herein were considered. Independent claims 1 and 30 have been voluntarily amended. Upon entry of this amendment, claims 1-39 and 49-53 are presently pending and under consideration (claims 40-48 are withdrawn).

Independent claims 1 and 30 have been voluntarily amended to more distinctly point out the characteristics and features of the claimed subject matter. More particularly, step (a) of claims 1 and 30 have been amended to clarify that the method of the invention pertains to isolating, from a "complex mixture of peptides comprising" post-translationally modified peptides from two or more different proteins, a particular target population of such modified peptides. This language is consistent with the preamble of these claims.

These amendments are supported throughout the specification and claims as originally filed, for example, at p. 6, lines 14-25; p. 6, line 26 to p. 7, line 18; p. 18, lines 5-17; p. 19, lines 13-20; p. 21 lines 5-19, and the Examples. The present amendments do not introduce new matter.

SUMMARY OF INTERVIEW

Applicants thank the Examiner (and her SPE) for the courtesy of the second in-person interview conducted on August 15, 2006. Also present at the interview, in addition to the Examiner and Applicants' attorney, were Supervisory Patent Examiner Long Le (Art Group 1641), Quality Assurance Specialist Bonnie Eyler (Art Group 1600), Dr. John Rush, Ph.D. (the first named inventor on the present application, and a person of skill in the art to which the invention pertains), and Mr. Andrew Warner, J.D. (a patent attorney assisting Applicants' attorney with this case).

During the interview, Applicants' attorney first expressed his concern that "piecemeal

examination" of this case has occurred (instead of compact examination as required by MPEP §707.07(g)) because the newly raised enablement rejection was not earlier presented (and was not based on any amendment by the Applicants), and because the Examiner had previously stated on the record that the claimed subject matter was free and clear of all prior arts. Applicants' attorney also discussed that the newly raised enablement rejection was improper on its face because the Examiner has failed to establish the *prima facie* showing required under the law and rules, and has instead relied only on opinion (rather than evidence, fact, and a Wands analysis) in making this rejection. SPE Lee and QAS Eyler acknowledged that a *prima facie* showing has not been made.

Applicants' attorney also pointed out that the newly cited references (Kanner *et al.* and Wirth *et al.*) do not anticipate or make obvious the presently claimed invention because they fail to teach, suggest, or enable *each and every element* of the claimed subject matter. More particularly, Applicants' attorney explained to the Examiner the fact that the cited references fail to disclose the selective isolation of a desired population of post-translationally modified *peptides* from a *complex mixture of peptides* (in contrast to immunoprecipitating *full length proteins* from a mixture *of proteins*, which is what the cited references teach). The Examiner was informed by Dr. Rush that the protein isolation methods disclosed in Kanner and Wirth (both of which involve tedious gel separation of proteins prior to determining their sequence) are well-known prior art techniques already distinguished by Applicants' in the Background of the specification, and are *not suitable nor enabling* for Applicants' method – a fact irrefutably established by the publications of record in this case (*see* references discussed below) that establish the state of the art prior to Applicants' invention.

In the interview, Applicants' attorney and Dr. Rush explained again to the Examiner the state and shortcomings of the art existing at the time the present application was filed, and how these shortcomings were solved by the method of the invention. Several prominent review articles (including four *already of record*) clearly establishing the state of the art were discussed. These references (discussed again in detail below) clearly evidence the *failures* of the prior art to provide a suitable method for the selective isolation of post-translationally modified peptides from complex mixtures of peptides as presently claimed, and explicitly *teach away* from the present invention. These references support the novelty and non-obviousness of the presently claimed subject matter, as well as establish that prior art methods for protein isolation – like those taught in Kanner and Wirth – *are not enabled or suitable for* isolating modified peptides from complex mixtures of peptides.

At the conclusion of the interview, Applicants' attorney discussed certain voluntary claim amendments (presented herein) that further point out the characteristics and features of the claimed

subject matter, and further distinguish the invention from prior art methods (including the limited teachings of Kanner and Wirth). SPE Le acknowledged that the methods disclosed in Kanner and Wirth are distinguished from the present invention and do not teach all elements of the claimed subject matter.

THE PROBLEM SOLVED BY THE INVENTION

In order to assist the Examiner's understanding of Applicants' invention as presently claimed, and to better appreciate how the cited prior art (Kanner and Wirth) is distinguished from Applicants' invention, the following overview of the problem solved by the present invention is provided.

The present invention is directed to methods suitable for the immunoaffinity isolation of a target population of naturally-occurring post-translationally modified *peptides* (*e.g.* acetylated or glycosylated peptides) from *a complex mixture of peptides*. As discussed at length in the Background of the specification (and with the Examiner at both in-person interviews), there was, at the time of Applicants' invention, an *unsolved need* in the art for such a method. At the time of Applicants' invention, certain prior art methodologies did exist which were suitable for isolating full length post-translationally modified *proteins* from a mixture of proteins (*e.g.* a cell extract). However, as established in the Background of the specification and in the state of the art references discussed below, these methods were *not* useful or suitable for the selective isolation of a population of post-translationally modified peptides. [1] In part, these prior art methods were unsuitable because, as discussed in the Background, they involved time-consuming and costly pre-purification steps of individual proteins (*e.g.* gel separation of proteins and then excision of individual bands before identification of protein identity or sequence, as taught by the cited Kanner and Wirth references) that rendered them not suitable for high-throughput protein modification site discovery (e.g. identification of all phosphorylation sites within a group of proteins).

Applicants' solved the problem by developing and providing, for the first time, a simple methodology suitable for the selective immunoaffinity isolation, from a complex mixture of *peptides*, a desired target population of naturally-occurring post-translationally modified *peptides*. The novel method allows essentially a one-step isolation of such target peptides and is readily coupled to the

[1] Indeed at least one of these reviews (Conrads *et al.*), published after the filing of the present application, expressly states that Applicants' invention solved the limitations of the unsuitable prior art protein isolation methodologies.

mass spectrometric identification of such isolated peptides, thus the method is suitable for high-throughput analyses, like global profiling of most if not all acetylation or phosphorylation sites within the proteome of a given cell. Applicants' method, having met a previously unsolved need in the art, has been *highly commercially successful* and in demand with proteomics-focused academic institutions and corporations alike. For example, to date, several prominent academic institutions, including U.C.L.A., Harvard, and M.I.T., are considering licensing Applicants' invention, and the Assignee of the present invention has conducted or is conducting pilot projects utilizing this invention for most of the major pharmaceutical companies, including ASTRAZENECA, NOVARTIS, and MERCK.

STATE OF THE ART – MANN, MARCUS, QUADRONI & CONRADS REFERENCES & OTHERS

During the interview, Applicants' attorney and Dr. John Rush again discussed several review articles (already of record in the case) establishing the state of the art at the time the present invention was filed. These references, which were previously discussed with the Examiner in the first in-person interview in this case, along with other prior art discussed in the Background of the specification, clearly evidence the novelty and non-obviousness of the presently claimed subject matter. Specifically, the following references were discussed: Mann *et al., Trends in Biotech. 20:* 261-268 (2002) (cited and discussed in the Background (Ref. CG)) (hereinafter "Mann"); Marcus *et al., Electrophoresis 21:* 2622-2636 (2000) (cited and discussed in the Background (Ref. CF); Quadroni *et al.*, "Phosphopeptide Analysis", *Proteomics in Functional Genomics 88:* 199-213 (2000) (Ref. DA of record); and Conrads *et al., Nat. Biotech. 23:* 36-37 (Jan. 2005) (Ref. CZ of record).

Mann (Ref. CG) is a review of peptide isolation and phosphoproteomic mass spectrometry approaches authored by one of the recognized leaders in the field to which the present invention relates. As discussed in the interview, this review represents and expressly states the prevailing view (at the time the present application was filed) that phospho-specific antibodies were *not suitable* for selectively isolating phosphorylated *peptides* from mixtures, due to various technical limitations, and *other methods must be used. See* Mann p. 261, last paragraph.

Marcus (Ref. CF) is an article describing the study of tyrosine-phosphorylated proteins using mass spectrometric techniques and anti-phosphotyrosine antibodies. As discussed in the interview (and noted in the Background of the specification at p. 4), the paper expressly concludes that the detection of phosphorylation sites (phosphopeptides) using such an antibody is "almost impossible" due to various technical limitations, including binding affinity. *See Marcus* p. 2635, end of 3.2.1.

Quadroni (Ref. DA) is a review article of phosphopeptide analysis techniques discussing and representing the state of the art around the time of the present invention. As discussed in the interview, the paper, in discussing the use of anti-phospho amino acid antibodies to immunoaffinity isolate phosphopeptides, expressly states that "As a general rule, all these antibodies behave quite poorly as affinity reagents, especially bad towards small peptides, and their main application remains in Western blotting." *See* Quadroni at p. 201, end of 1.3. The paper also expressly states that attempts to use anti-phosphotyrosine antibodies to isolate phosphotyrosine-containing peptides had failed.

In the interview, Dr. Rush re-emphasized that the present invention was in fact a novel and surprising advance over prior approaches, since the prior art, including publications like Mann, Marcus, and Quadroni, clearly *taught away* from the suitability of using post-translational modification-specific antibodies to immunoaffinity isolate target post-translationally modified peptides from complex mixtures of peptides. As also discussed in the interview, the novelty of the present invention is underscored by the surge of commercial and academic interest in Applicants' invention, as discussed above.

Dr. Rush and Applicants' attorney also discussed, during the interview, that the novelty of the present invention is further underscored by Conrads (Ref. CZ), a review article of Applicants' method that appeared in the leading journal, *Nature Biotechnology*, shortly after Applicants published an article about their method (following the filing of this application). In the review, the authors expressly conclude that the Applicants' invention "address[es] the deficiency" in prior art proteomics approaches for isolating phosphopeptides, and go on to highlight several of the problems (*e.g.* low abundance of phosphopeptides from complex mixtures, need for enrichment, etc.) with prior art approaches that remained unsolved until the Applicants' invention.

REINDERS, PETERS, AND RAGGIASCHI REFERENCES

In addition to the references of record discussed above, Applicants' attorney and Dr. Rush also provided to the Examiner and discussed during the interview three additional references that further evidence the novelty and non-obviousness of the claimed subject matter over prior art protein isolation methods. These three references (all of which were written and/or published after Applicants' own publication of their method) are being concurrently filed by Supplemental IDS as Refs. DB, DC, and DD, respectively.

Reinders *et al.*, *"State-of-the-art in Phosphoproteomics," Proteomics 5: 5042-4061* (2005)

(Ref. DB) is a review of phospho-protein and phospho-peptide isolation and analysis approaches authored by one of the leaders in the field to which the present invention relates. As discussed in the interview, this review represents and expressly states the prevailing view that while phospho-specific antibodies have historically been used to immunoprecipitate phosphorylated *proteins*, these antibodies and approaches have *not been suitable* for selectively isolating phosphorylated *peptides* from mixtures, and *other methods have to be applied. See* Reinders at p. 4054, first pargraph.

Peters *et al., "Exploring the Phosphoproteome with Mass Spectrometry," Mini. Rev. Med. Chem. 4: 313-324* (2004) (Ref. DC) is another review of phospho-protein and phospho-peptide isolation and MS analysis approaches in the field to which the present invention relates. As discussed in the interview, this review also represents and expressly states the prevailing view that phospho-specific antibodies have been used to immunoprecipitate phosphorylated *proteins* (which then must typically be separated on a 2-gel before analysis), these antibodies and approaches have *not been suitable* for selectively isolating phosphorylated *peptides* from mixtures, due to various technical difficulties. *See* Peters at p. 314, second and third paragraphs. In fact, this review sets the stage for the value and novelty of Applicants' method by futuristically stating that "a potentially more powerful approach [to direct phospho-peptide isolation from complex mixtures] would involve the active enrichment of phosphopeptides."

Lastly, Raggiaschi *et al., "Phosphoproteome Analysis," Biosci. Reports 25(1/2): 33-44* (2005) (Ref. DD) is another review of techniques for analyzing phospho-proteins and phospho-peptides on a proteome-wide basis. As discussed in the interview, this review distinguishes between classical methods of isolating phosphorylated proteins (*e.g.* by using phospho-tyrosine specific antibodies to immunoprecipitate them) as opposed to phosphorylated *peptides*, and emphasizes that in order to broadly identify phosphorylation sites it is necessary to isolate phospho-*peptides* (because merely isolating phospho-*proteins*, each of which may have multiple unique phosphorylation sites, is not adequate to identify the phospho-sites). *See* Raggiaschi at p. 35, first paragraph, and pgs. 34-41 generally.

All of the above-discussed references irrefutably evidence the following fact: prior to the instant invention, while certain methods of immunoprecipitating full length phospho-proteins were known in the art, those methods were not suitable nor enabling for the selective isolation of desired post-translationally modified peptides, such as phospho- or acetyl-peptides, from complex mixtures of peptides. The development of a suitable selective peptide isolation and characterization method remained an unsolved need in the art until the time of Applicants' invention.

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§112, 2ND PARAGRAPH, ENABLEMENT REJECTIONS

The Examiner has newly rejected claims 1-39 and 49-53 under 35 U.S.C. §112, second paragraph, as allegedly lacking enablement. The Examiner asserts that one of skill in the art to which the invention pertains would not be able to practice the claimed invention without undue experimentation because Applicants' specification does not include working examples utilizing antibodies other than phospho-specific antibodies (*see* June 14, 2006 Office Action at p. 3).[2] Applicants submit the rejection is improper, not supported by any evidence, and should be withdrawn.

As an initial matter, the present enablement rejection is improper under principles of compact prosecution and avoidance of "piecemeal examination" (*see* MPEP §707.07(g)) because it is raised for the *first time* late in prosecution (in the *third* Office Action in this case), following not only an in-person interview but a Final Office Action -- both of which provided the Examiner an opportunity to raise any concerns about enablement -- in which it was not raised.[3]

a. The Examiner has Failed to Establish the Required *Prima Facie* Showing of Lack of Enablement.

It is well established that an application as filed *must* be taken as being enabling for a claimed invention unless an Examiner establishes otherwise. *See* MPEP §2164.04, *citing In re Marzocchi, 169 U.S.P.Q 367* (CCPA 1971); *see also In re Brana, 34 U.S.P.Q.2d 1436 (Fed. Cir. 1995). The initial burden is on the Examiner* to present *evidence or fact-based reasons* why a person skilled in the art would not be able to *make and use* the claimed invention without undue experimentation. *See* MPEP §2164.04.

An Examiner must carry this burden with findings of fact and/or factors and reasoning sufficient to support an allegation of inadequate enablement. *See* MPEP §2164.04. It is well established that a general allegation of non-enablement based only on the opinion of an Examiner is *not* a sufficient reason to make or support an enablement rejection. *See* MPEP §2164.04, *citing In re Marzocchi, Id.*; *In re Wright*, 27 U.S.P.Q.2d (Fed. Cir. 1993).

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[2] Oddly, included in this rejection are previously-allowed claims 30-33 and 36-39, which are directed to isolation of phospho-peptides, despite the fact that the specification contains nine working examples of the isolation of phospho-peptides.

[3] Indeed, the Examiner has also presently rejected claims 30-33 and 36-39 as lacking enablement despite having found those claims *enabled and allowed* in the prior Office Action (those claims were in no way amended by Applicants' after their allowance).

In the present case, the Examiner has failed to provide *any* evidence or facts (much less a preponderance) supporting why one of ordinary skill in the mature and developed art of post-translational protein/peptide isolation and characterization would not be able to *make and use* the claimed method without undue experimentation. No express findings of *fact* have been presented. No *evidence or supportable reasoning* has been adduced to support the cursory allegation that the claimed invention is not enabled. Indeed, the required *prima facie* showing is not possible based on the mature state of this art, the detailed teachings of the specification, and the evidence of record in this case (including review articles of post-translational proteomic methods).

Accordingly, the Examiner has erred by failing to establish the required *prima facie* showing of lack of enablement. The new rejection of claims 1-39 and 49-53 is therefore improper, and should be withdrawn.[4]

b. The Examiner has Misapplied a Working Examples Requirement that Does *not* Exist under U.S. Patent Law or Rules.

It is firmly and clearly established under U.S. patent law that the presence or absence of working examples, or any particular number of working examples, is not the test of whether a claimed invention is adequately enabled. *See* MPEP §2164.02.[5] Rather, what the patent law requires is determining whether a skilled artisan can make and use the invention as claimed without undue experimentation, based on both the teachings of the specification and the general knowledge in the relevant art. This determination is fact-based and must consider a variety of factors, as the Federal Circuit Court of Appeals made clear in *In re Wands*, 858 F.2d 731 (Fed. Cir. 1998); *see also* MPEP §2164.01(a). These Wands factors (as they are widely known) include:

(1) The quantity of experimentation required given the content of the specification;

(2) The amount of direction/guidance provided in the specification;

(3) The presence or absence of working examples;

(4) The nature of the invention;

(5) The state of the prior art;

(6) The relative skill of those in the art;

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[4] Indeed, this fact was acknowledged by SPE Le and QAS Eyler in the second in-person interview held on August 15, 2006.

[5] In fact, no working examples are required at all as long as the skilled artisan can practice the claimed invention without an undue amount of experimentation. *See* MPEP §2164.02.

(7) The level of predictability in the art; and

(8) The breadth of the claims.

Among these factors, the amount of guidance provided in the specification and the level of skill and knowledge in the relevant art are particularly important. *See* MPEP §2164.03, *citing In re Fisher*, 166 U.S.P.Q. 18 (CCPA 1970). Again, the ultimate test in considering the various Wands factors remains clear:

> "As long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. §112 is satisfied." MPEP §2164.01(b), *citing In re Fisher, Id.*

In the present case, the Examiner has nonetheless misapplied a number of working examples test to Applicants' specification and claimed invention, and has improperly based the new enablement rejection on nothing more than the absence of actual working examples *for each preferred species* of antibody recited in the claims. The Examiner has utterly failed to consider the Wands factors, has provided *absolutely no other basis* for why the 133-page specification, as filed, would not enable the skilled artisan to *make and use* the claimed invention without undue experimentation. This error is all the more striking considering that the specification does in fact contain many detailed working examples employing various phosphorylation-specific antibodies, as well as detailed prophetic example teaching how to employ an acetylation-specific antibody in the claimed method.

Accordingly, the Examiner has erred by failing to apply the proper enablement analysis and misapplying an improper presence/absence of particular working examples test to support an assertion of inadequate enablement. The new enablement rejection of claims 1-39 and 49-53 is therefore improper, and should be withdrawn.

c. Consideration of the *Wands* Factors Irrefutably Leads to a Conclusion that the Claimed Invention is Sufficiently Enabled.

To satisfy the enablement requirement, a specification must teach one of skill in the art to which the invention pertains to make and use the claimed invention without undue experimentation. MPEP §2164.01. As long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement is satisfied. MPEP §2164.01(b), *citing In re Fisher, Id.*

The enablement analysis *must* be conducted from the viewpoint of persons skilled in the field of the invention at the time the patent application was filed. *See Ajinomoto Co., Inc. v. Archer-Daniels-Midland Co.*, 56 U.S.P.Q.2d 1385 (Fed. Cir. 2000). The determination is fact-based and must consider a variety of factors, generally known as the Wands factors. MPEP §2164.01(a), *citing In re Wands, Id.* These factors are each discussed at length below.

1. *The Nature of the Invention and The Breadth of Claims.*

The invention as claimed is directed to a method for employing post-translational modification-specific antibodies in immunoaffinity format to selectively isolate, from a complex mixture of peptides, a desired target population of peptides containing a particular post-translationally modified amino acid or motif. The population of modified peptides so isolated may then be characterized by mass spectrometry and further by database searching in order to identify the parent proteins from which such modified peptides are derived, thus providing a global snapshot of protein modification in a given cell at a given time.

In order to make and use the invention as most broadly claimed (claim 1), a skilled artisan need only be able (without undue experimentation) to immobilize a post-translational modification specific-antibody in immunoaffinity format and use such immobilized antibody to selective isolate a desired population of modified peptides from a complex mixture (and, subsequently, if desired, characterize the isolated peptides by mass spectrometry). As discussed in the next section, the artisan of skill in the mature and well-developed arts of antibody use, peptide/protein isolation techniques, and mass spectrometry techniques can *readily* practice these steps without undue experimentation.

Indeed, this fact is clearly evidenced by an article (Conrads *et al.*, Ref. CZ, of record) reviewing Applicants' novel method (as published by inventor Rush *et al.*, in the journal Nature Biotechnology) and other proteomics methods in the field, which states:

> "The elegance of the study by Rush *et al.* is that it uses *readily available reagents* and *does not require any sophisticated chemistry or chromatography* . . ." Conrads at p. 36, last full paragraph (emphasis added).

2. *The Mature State of the Art and its High Level of Skill.*

The Federal Circuit Court of Appeals, the USPTO Board of Appeals, and the USPTO itself have all recognized that the production and use of antibodies (including in various immunoaffinity

techniques) is a mature field with a very high level of skill. For example, in its Revised Interim Written Description Guidelines (http://www.uspto.gov/web/ patents/ guides.htm), the USPTO discusses exemplary antibody claims and technology and clearly states:

> "*This is a mature technology where the level of skill is high and advanced . . . antibody technology is well developed and mature . . .*" *See* Written Description Guidelines at p. 59-60 (emphasis added).

The Federal Circuit, in favorably commenting on the USPTO's Guidelines, acknowledged the mature nature of the antibody art its high level of skill and knowledge in *Enzo Biochem, Inc. v. Gen-Probe, Inc.*, 296 F.3d 1316 (Fed. Cir. 2002). Other Federal Circuit decisions have also recognized the mature nature of antibody production, characterization and use, and the high level of skill in this art. *See, e.g. Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367 (Fed. Cir. 1986). The USPTO Board of Patent Appeals too has explicitly recognized the mature state of this art. *See, e.g. Ex Parte D*, 27 USPQ2d 1067 (BPAI 1993).

Beyond this evidence, "Patents and printed publications in the art should be relied upon to determine whether an art is mature and what the level of knowledge and skill is in the art." *See In re Hayes Microcomputer Products, Inc. Patent Litigation, 982* F.2d at 1527 (Fed. Cir. 1992).

In the present case, the printed publications of record in this case (Refs. CG, CF, CZ and DA-DD, discussed at length above), which are mostly reviews of the state-of-the-art and techniques in the field of modified peptide and protein isolation to which the invention relates, all evidence that is a mature art, where the level of skill and knowledge is very high. The advanced knowledge in this art strongly supports that claimed invention, as described in the detailed 133-page specification as filed, is sufficiently enabled.

3. *The Detailed Guidance & Working Examples Provided in the Specification.*

Against this backdrop of extensive knowledge and skill in this mature art, the Applicants' filed a 133-page, very detailed specification describing their invention (as presently claimed): a new and powerful method for quickly isolating, and optionally characterizing, a target population of naturally-occurring post-translationally modified peptides from a complex mixture of peptides (the complex mixture, for example, potentially also containing many other types of modified peptides and unmodified peptides that are not desired to be isolated).

The claimed invention, in its most broad sense (claim 1, as presently amended), is a method

for isolating a population of naturally-occurring post-translationally modified peptides from a complex mixture of peptides, said method comprising the steps of:

(a) obtaining a proteinaceous preparation from an organism, wherein said proteinaceous preparation comprises a complex mixture of peptides comprising naturally-occurring post-translationally modified peptides from two or more different proteins;

(b) contacting said proteinaceous preparation with at least one immobilized post-translational modification-specific antibody; and

(c) isolating at least one population of naturally-occurring post-translationally modified peptides specifically bound by said immobilized modification-specific antibody in step (b).

In a preferred embodiment, the claimed invention further comprises the step of:

(d) characterizing said population of modified peptides isolated in step (c) by mass spectrometry (MS), tandem mass spectrometry (MS-MS), and/or MS^3 analysis.

The 133-page specification provides *detailed* guidance to the skilled artisan on how to practice *each* of these required steps.

Obtaining a Complex Mixture of Peptides.

The specification has an *entire section* entitled "Proteinaceous Preparations" (pgs. 23-28) providing detailed guidance and how to obtain/prepare a complex mixture of peptides, which will then be used in immunoaffinity isolation to obtain the desired target population of post-translationally modified peptides. This detailed guidance teaches a skilled artisan the types of organisms, tissues, and cells from which proteinaceous preparations may be desirably obtained (pgs. 23-25), how to obtain a complex mixture of peptides from such preparations (pgs. 25-28), and the types of post-translationally modified peptides (including "phosphorylation, acetylation, methylation" and others) contained in such complex mixtures that may be suitably isolated by the method of the invention (p. 26). The specification also provides, on p. 21, express definitions of what "modified peptides", "complex mixtures of peptides", and "proteinaceous preparations" mean with the context of the method of the invention.

Immunoaffinity Isolation of Target Peptides Using Modification-Specific Antibodies.

The specification also has *entire sections* entitled "Immunoaffinity Isolation" (pgs. 28-36) and "Modification-specific Antibodies" (pgs. 36-42) providing detailed guidance on the types of modification-specific antibodies that may be employed in the invention, and how to immobilize them for use in immunoaffinity capture of desired post-translationally modified peptides.

This detailed guidance teaches a skilled artisan how to prepare modification-specific (including motif-specific) antibodies against any desired post-translationally modified amino acid or motif (pgs. 36-42). The artisan is taught, on p. 36, that some preferred antibodies are those that bind modified peptides comprising "phosphorylation, acetylation, methylation, nitrosylation, glycosylation, etc." The specification, on p. 36, cites to Comb *et al.* (WO 00/14536, "Production of Motif-Specific and Context-Independent Antibodies Using Peptide Libraries as Antigens"), a publicly available reference (now issued as U.S. Patent No. 6,441,140) teaching the skilled artisan how to produce and use such antibodies. The specification also provides detailed guidance on the actual steps of the Comb *et al.* antibody production method (*see* pgs. 38-42).

The detailed guidance provided in the specification also teaches a skilled artisan how to immobilize antibody on various supports suitable for immunoaffinity capture (pgs. 28-29), how to conduct the actual immunoaffinity capture of the target modified peptides (pgs. 29-34), and how additional chromatography steps may optionally be carried out if desired (pgs. 34-36).

At the time the present specification was filed, a skilled artisan could actually commercially buy (rather than having to produce) many modification-specific antibodies useful in the methods of the invention, including but not limited to phosphotyrosine-specific antibodies, acetyl-lysine-specific antibodies, nitro-tyrosine-specific antibodies, and various phosphorylated kinase consensus substrate and protein-protein binding motif-specific antibodies (*see, e.g.*, Cell Signaling Technology, Inc., 2000-2001 Catalogue pgs. 11-27 (submitted herewith for the Examiner's convenience)). Other post-translational modification-specific antibodies useful in the method of the invention, if not commercially available at the time the present application was filed, could readily and simply be produced by the skilled artisan according to the teachings of the specification and the method of Comb *et al.* cited therein.[6]

Identification of Peptides By Mass Spectrometry.

The specification also has an *entire section* entitled "Analysis of Isolated Peptides" (pgs. 43-56) providing detailed guidance on how to analyze the target population of post-translationally modified peptides that have been immunoaffinity isolated from the complex peptide mixture. This detailed guidance teaches a skilled artisan how to carry out various forms of mass spectrometric

[6] The USPTO has already found this antibody production method (and the class of antibodies), novel, enabled and patentable. *See* U.S. Patent Nos. 6,441,140; 6,982,318 and USSN 10/014,485 (allowed); USSN 10/139,841 (allowed).

analysis of isolated peptides (pgs. 43-50), and how to subsequently use database searching to identify the parent proteins of such modified peptides (pgs. 50-53).

Working Examples.

Beyond the detailed guidance discussed above, the specification also provides no less than *nine* detailed working examples (Examples I-VII and XI-XII, pgs. 63-112 and 119-124) and another *three* detailed prophetic examples (Examples VIII-X, pgs. 113-118) that further teach the skilled artisan how to make and use the claimed invention. These working examples describe in detail how to carry out each of the steps required in the practice of the invention (as most broadly claimed in claim 1), and include one explicit example (Example IX, pgs. 114-116) of how to practice the method for the isolation of acetylated-peptides. Indeed, skilled artisans have in fact been practicing Applicants' method as described in the specification to isolate acetylated-peptides from complex mixtures of peptides (*see, e.g.*, Kim *et al.*, *Molec. Cell 23:* 607-618 (2006)), and the inventors have also employed their method using acetyl-specific antibodies to identify hundreds of novel protein acetylation sites.

Clearly, then, the specification provides a *high level* of guidance to the skilled artisan in how to make and use the claimed invention, further supporting that the invention is enabled.

4. *The Quantity of Experimentation Required Given the Detail of the Specification.*

Given the nature of the invention, the mature nature of the art to which the invention relates and the very high level of those of skill in this art, and the detailed guidance and examples provided by Applicants in their specification, there is *little* experimentation (much less undue experimentation) required for the skilled artisan to make and use the invention as claimed. All such an artisan need do to make and use the claimed invention is follow the teachings of the specification and employ their knowledge of well-known art techniques such as antibody preparation and use, and immunoaffinity techniques, to carry out the required steps of the claimed method (as most broadly set out in claim 1).

Indeed, as noted above, the ease with which the skilled artisan can make and use the claimed invention has already been acknowledged in the art by the Conrads *et al.* review publication (Ref. CZ, of record), which states:

> "The elegance of the [method] is that it uses *readily available reagents* and *does not require any sophisticated chemistry or chromatography . . .*" Conrads at p. 36, last full paragraph (emphasis added).

In conclusion, conducting the required enablement assessment by considering the *Wands* factors, consulting the publications of record establishing the state of the art in this field, and reading the detailed 133-page specification through the eyes of a skilled artisan inevitably leads to the following conclusion: One of ordinary skill in the art would readily be able to make and use, *without undue experimentation*, the presently claimed method for the immunoaffinity isolation of a target population of naturally-occurring post-translationally modified peptides from a complex mixture of peptides. The specification therefore satisfies the enablement requirement.

Accordingly, the Examiner's unsupported assertion to the contrary and the outstanding enablement rejections of claims 1-39 and 49-53 are improper and unsustainable, and should be withdrawn.

NOVELTY REJECTIONS -- BASED ON KANNER AND WIRTH

The Examiner has newly rejected claims 1, 10, 13-16, and 19 under 35 U.S.C. §102(b) as allegedly being anticipated by Kanner *et al.*, *J. Immunol. Meth. 120:* 115-124 (1989) (hereinafter "Kanner")). The Examiner asserts that Kanner disclose the selective immunoaffinity isolation of post-translationally modified peptides from complex mixtures of peptides using phospho-tyrosine-specific antibodies, and thus renders the claimed invention not novel (*see* June 14, 2006 Office Action at p. 4).

The Examiner has also newly rejected claims 1, 2, 4, 5, 10, and 13-23 under 35 U.S.C. §102(b) as allegedly being anticipated by Wirth *et al.*, *Electrophoresis 14:* 1199-1215 (1993) (hereinafter "Wirth")). The Examiner asserts that Wirth disclose the selective immunoaffinity isolation of post-translationally modified peptides from complex mixtures of peptides using phospho-tyrosine-specific antibodies, and thus renders the claimed invention not novel (*see* June 14, 2006 Office Action at p. 5).

Applicants respectfully disagree, as neither reference in fact teaches, suggests, or enables a method of selective isolating a target population of modified peptides from a complex mixture of peptides, but instead merely disclose methods for immunoprecipitating modified proteins from a protein mixture. The Examiner's misunderstanding of this important difference between Applicants' method and prior art methods, including those of Kanner and Wirth, was discussed in detail during the second in-person interview in this case.

APPLICANTS: Rush *et al.*
U.S.S.N.: 10/777,893

I. *Both Kanner and Wirth Fail to Teach Each and Every Element of the Claims.*

It is a bright-line rule that a cited reference *only* anticipates a claimed invention if the reference discloses *each and every element or limitation* of the subject matter *as claimed*; the so-called "all elements" rule. *See* MPEP §706.02; MPEP §2131, *citing Verdegaal Bros. v. Union Oil of Cal.* (Fed. Cir. 1987). The Examiner *bears the burden* of establishing that a single reference in fact discloses each and every element, must consider the reference as whole, and may *not* read teachings into a reference that do not exist. MPEP §2131. The Kanner and Wirth references fail to meet this test because they do not teach each and every element or limitation of the claimed subject matter.

As a starting point, the Examiner must consider language of the claims to determine what elements are required, giving the claims terms their literal meaning, unless defined/used otherwise in the specification. MPEP §2111.01, *citing In re Zletz*, 13 U.S.P.Q.2d 1320 (Fed. Cir. 1989). The literal meanings of claim terms in considered *through the eyes of those of skill in the art* to which the invention pertains.

The required elements and limitations of the presently claimed invention, in its broadest sense, are set out in claim 1 (as previously presented), which reads:

A method for isolating a population of naturally-occurring post-translationally modified peptides from a complex mixture of peptides, said method comprising the steps of:

(a) obtaining a proteinaceous preparation from an organism, wherein said proteinaceous preparation comprises a complex mixture of peptides comprising naturally-occurring post-translationally modified peptides from two or more different proteins;

(b) contacting said proteinaceous preparation with at least one immobilized post-translational modification-specific antibody; and

(c) isolating at least one population of naturally-occurring post-translationally modified peptides specifically bound by said immobilized modification-specific antibody in step (b).

Read literally, the claims require that a population of post-translationally modified peptides be specifically isolated from a preparation containing post-translationally modified peptides from two or more different proteins. Literally, the claims are not directed to, nor encompass, the isolation of proteins from mixtures of proteins. This claim interpretation is consistent with and supported by the entire teaching of the 133-page specification, which makes clear that the method of the invention isolates *peptides* (not proteins). Indeed, an explicit definition of peptide is provided in the specification:

" 'peptide' means a fragment of a whole protein, e.g. a protease cleavage fragment, having a

sequence two or more amino acids long." p. 21, lines 5-6.

Similarly, an explicit definition of a complex mixture of peptides is also provided in the specification:

" 'complex mixture of peptides' means a substantially unpurified mixture of a plurality of different peptides corresponding to two or more different proteins, typically including both modified and unmodified peptides." p. 21, lines 16-19.

Elsewhere, the specification makes this distinction clear, for example:

"The method of the invention enables the single-step isolation (and subsequent characterization) of multiple different peptides, corresponding to a multitude of different proteins . ." p. 19, lines 13-16.

In other words, it is crystal clear to the skilled artisan that a peptide is not a protein (but is *derived from* a protein) and that the claimed invention pertains to the selective isolation of *peptides*, not proteins. The difference is important, because as noted in Mann et al. (Ref. CG) and Raggiaschi *et al.* (Ref. DD), discussed above, in order to get a truly global snapshot of, *e.g.*, protein phosphorylation in a cell, one must be able to isolate and identify a great number of phosphorylated *peptides* (not proteins), since merely isolating the proteins would fail to identify the many different phosphorylation sites occurring on those proteins.

Turning to Kanner and Wirth, both references disclose no more than the purification of tyrosine phosphorylated *proteins* from mixtures of *proteins* using well-known prior art techniques. [7] Kanner is entitled "Immunoaffinity purification of tyrosine-phosphorylated cellular proteins," and discloses that tyrosine-phosphorylated proteins in a cell extract may be immunoprecipitated using a phosphotyrosine-specific antibody, and then separated by gel electrophoresis prior to further analysis. Wirth is entitled "The rat liver epithelial (RLE) cell nuclear protein database," and discloses a similar approach. Both references completely fail to disclose the isolation of modified peptides from a complex mixture of peptides, as required by the claimed subject matter. Neither references in any way discloses that the cell extracts studied in fact contained "post-translationally modified *peptides from two or more different proteins*" as required by step (a) of Applicants' claimed method.[8] Neither

[7] The Examiner may have been misled by the fact that these references use the term "protein" interchangeably with the term "polypeptide" (neither of which is a peptide).

[8] Moreover, there is no evidence of record to support an argument that the whole protein cell extracts of Kanner and Wirth inherently must contain modified peptides from two or more different parent proteins. *See* MPEP §2112(IV). However, even if that were shown to be true, Kanner and Wirth would still fail to teach the selective isolation of such peptides, as required by step (c) of the claims.

reference in any way discloses "*isolating a least one population* of naturally-occurring post-translationally modified *peptides*" as required by step (c) of Applicants' claimed method.

Rather, as discussed at length in the second in-person interview with the Examiner, Kanner and Wirth disclose no more than traditional and well-known prior art immunoprecipitation methods that, while useful for studying modified *proteins*, are recognized as not being suitable for isolating modified *peptides*. The limitations and unsuitability of prior art approaches like that disclosed in Kanner and Wirth is discussed at length in the Background section of the specification, and is expressly evidenced by all of the proteomics techniques review articles that have been cited, discussed, and made of record in this case (*see* Refs. CG, CF, CZ and DA-DD). In fact, one such reference, Conrads *et al.* (Ref. CZ) clearly states that Applicants' method is new, powerful, elegant, and solves the limitations of the prior art approaches. Indeed, if Kanner and Wirth had in fact disclosed the presently claimed subject matter, Applicants would not have experienced the surge of commercial and academic interest in the present invention that has in fact occurred.

Since Kanner and Wirth fail to teach each and every element and limitation of the claimed method, the presently claimed subject matter is novel and patentable over these references (and all other prior art).[9] Accordingly, the novelty rejection of claims 1, 2, 4, 5, 10, and 13-23 is improper and should be withdrawn.

Nonetheless, Applicants have presently voluntarily amended independent claims 1 and 30 in order to more distinctly point out the characteristics and features of the claimed invention; namely, that the proteinaceous preparation of step (a) comprises "a complex mixture of" the recited modified peptides. As discussed with the Examiner in the second in-person interview, this amendment makes explicitly clear in step (a) of the claims what is already clear from the both the preamble of the claims and the teachings of the specification; namely, that the proteinaceous preparation (which will be contacted with the immobilized antibody) contains a complex mixture of peptides. *See, e.g.* specification at p. 28, lines 12-16: "The proteinaceous preparation, [] contains a complex mixture of modified and unmodified peptides from a plurality of different proteins . . ."

This amendment further distinguishes the novelty of the claimed method over Kanner and

[9] SPE Le acknowledged the same in the in-person interview. Furthermore, since these references fail to meet all elements of the broadest independent claims, they also fail to meet all elements of any of the dependent claims. In some instances, the Examiner has improperly rejected dependent claims with elements that are simply not taught at all in the cited references (for example, Wirth is devoid of any teachings relating to mass spectrometric identification of peptide sequence, despite the Examiner assertion to the contrary (see June 14, 2006 Office Action at p. 5).

Wirth (and all other prior art), as those references fail to teach contacting a complex mixture of peptides with an immobilized antibody.

II. *Neither Kanner or Wirth is Enabling for the Claimed Method.*

It is also a bright-line rule that a cited reference *only* anticipates a claimed invention if the reference is *enabling* for the subject matter *as claimed*. *See* MPEP §2121.01, *citing In re Hoeksema*, 158 U.S.P.Q. 596 (CCPA 1968). Stated another way, "A §102(b) reference must sufficiently describe the claimed invention to have placed the public in possession of it." *Paperless Accounting, Inc. v. Bay Area Rapid Transit Sys.*, 231 U.S.P.Q 649 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 933 (1987). The test for determining whether a prior art reference is enabled for a claimed invention is the same test for determining whether a specification is enabled: the cited reference must teach the invention in sufficient detail to enable one of skill in the art *to make and use it without undue experimentation*. *Minnesota Mining & Mfg. Co. v. Chemque, Inc.*, 64 U.S.P.Q.2d 1270 (Fed. Cir. 2002).

Turning to the disclosures of Kanner and Wirth, not only do both references fail to teach and every element of the claimed subject (as previously discussed), but both references further fail to enable Applicants' claimed method. Both references utterly fail to provide any teaching (much less detailed teaching) on the conditions and steps necessary to successfully selectively immunoaffinity isolate a population of post-translationally modified *peptides* from a complex mixture of *peptides*. Rather, their disclosures are limited to describing how full-length *proteins* may be immunoprecipitated (and subsequently separated by tedious gel separation) from a mixture of *proteins*, which techniques are *not* suitable for isolating peptides in accordance with the claimed method. Given the limited teachings of Kanner and Wirth, a person of skill in this art would, at the time the present application was filed, have needed to resort to *extensive and undue* experimentation to attempt to selectively isolate *peptides* using the method disclosed by those references.

Indeed, the best evidence of this undue experimentation and the fact that Kanner and Wirth are not enabling are the publications of record in this case that firmly establish *the failed prior attempts* to accomplish what Applicants' method accomplished for the first time, and which clearly state that prior art protein immunoprecipitation methods *don't work* for selectively isolating peptides. *See* Refs. CG, CF, CZ and DA-DD (discussed at length above).

For example, Mann (Ref. CG) expressly states the prevailing view (at the time the present application was filed) that phospho-specific antibodies were *not suitable* for selectively isolating

phosphorylated *peptides* from mixtures, due to various technical limitations. *See* Mann p. 261, last paragraph. Marcus (Ref. CF) expressly concludes that the detection of phosphorylation sites (phosphopeptides) using such phospho-specific antibodies is "almost impossible" due to various technical limitations, including binding affinity. *See Marcus* p. 2635, end of 3.2.1. Quadroni (Ref. DA) expressly states that "As a general rule, all these antibodies behave quite poorly as affinity reagents, especially bad towards small peptides, and their main application remains in Western blotting." *See* Quadroni at p. 201, end of 1.3. The paper also expressly states that attempts to use anti-phosphotyrosine antibodies to isolate phosphotyrosine-containing peptides had failed. The other references of record underscore the same point.

Accordingly, Kanner and Wirth both fail to anticipate the claimed method because these references fail to enable it. The novelty rejection of claims 1, 2, 4, 5, 10, and 13-23 is therefore improper and should be withdrawn.

OBVIOUSNESS REJECTIONS -- BASED ON WIRTH, LITTLE, PIDGEON, AND STOUGHTON

The Examiner has further rejected claims 3, 6-9, 11, 24-28, 30-39, and 49-52 under 35 U.S.C. §103(a) as allegedly being obvious given Wirth *et al.* (*Electrophoresis* (1993), *supra.*) in view of Little *et al.* (U.S. Pat. No. 6,322,970, (issued Nov. 27, 2001) (hereinafter "Little") – already of record in this case). The Examiner asserts that while Wirth fails to disclose certain additional aspects of preferred embodiments of Applicants' method (which are recited in the rejected dependent claims), Little teaches or suggests these steps, hence the claimed subject matter is obvious.

The Examiner has also rejected claim 12 under 35 U.S.C. §103(a) as allegedly being obvious given Wirth *et al.* (*supra.*) in view of Little *et al.* (*supra.*) and in further view of Pidgeon *et al.* (U.S. Pat. No. 6,579,720, (issued June 17, 2003) (hereinafter "Pidgeon")– already of record in this case). The Examiner asserts that while Wirth fails to disclose the preferred embodiments of Applicants' method (which are recited in rejected dependent claim 12 and claim 11), Little combined with Pidgeon teach or suggest these steps, hence the claimed subject matter is obvious.

Lastly, the Examiner has also rejected claim 29 under 35 U.S.C. §103(a) as allegedly being obvious given Wirth *et al.* (*supra.*) in view of Little *et al.* (*supra.*) and in further view of Stoughton *et al.* (U.S. Pat. No. 5,965,352, (issued October 12, 1999) (hereinafter "Stoughton") – already of record in this case). The Examiner asserts that while Wirth fails to disclose the preferred embodiments of Applicants' method (which are recited in rejected dependent claim 29 and claim 25), Little combined with Stoughton teach or suggest these steps, hence the claimed subject matter is

obvious.

Applicants respectfully disagree and submit that the Examiner has failed to establish the required *prima facie* showing of obviousness. Further, the deficiencies of the primary reference, Wirth, are in no way cured by the limited teachings of Little, Pidgeon, and/or Stoughton.

It is axiomatic that an Examiner must establish *a prima facie* case of obviousness by establishing three elements: (i) that there is some suggestion or motivation in the references themselves – or if not, then in the knowledge generally available to those of skill in the art – to combine the teachings of the references; (ii) that there is some reasonable expectation of success, as evidenced by the cited references and/or other prior art, in so combining the teachings, and (iii) that the cited references teach or suggest each and every limitation of the claimed subject matter. *See* MPEP §§2142, 2143, *citing, e.g. In re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991). The mere fact that references can be combined is *not* sufficient to establish desirability or motivation to do so (*see* MPEP §2143.01, citing *In re Mills*, 916 F.2d 680) (Fed. Cir. 1990)).

In the present case, a *prima facie* showing of obviousness has not been established because none of the three required elements has been met. As discussed in the interview, the primary reference, Wirth, completely fails to teach, suggest, or make obvious Applicants' invention as presently claimed. The limited teachings of Wirth, and how the phospho*protein* immunoprecipitation method it discloses is distinguished from the present invention, have been described in detail above. Not only is there no teaching or suggestion of Applicants' method in Wirth, but a skilled artisan would have absolutely *no motivation or expectation* that its teachings could be applied to successfully isolate modified *peptides* from complex mixtures of *peptides*. In fact, such artisan would have had an *expectation of failure* given the clear prior art failures and teaching away evidenced by the references of record in this case (Refs. CG, CF, CZ and DA-DD, discussed above). Since the primary reference, Wirth, fails to render obvious the invention as most broadly claimed in independent claims 1 and 30, the claim rejections based on combinations of Wirth and the secondary references Little, Pidgeon, and Stoughton similarly fail. Accordingly, the subject matter of claims 3, 6-9, 11, 12, 24-29, 30-39, and 49-52 (as well as all other pending claims) is non-obvious and patentable over Wirth and the cited secondary references, and the rejection of these claims should be withdrawn.

Further, none of the secondary references – Little, Pidgeon, and Stoughton (nor any other references cited by the Examiner in this case) – taken alone or combined with Wirth, teach, suggest, or make obvious the presently claimed methods for selectively isolating, from a complex mixture of

peptides, a population of naturally-occurring post-translationally modified *peptides* by immunoaffinity isolation using a post-translational modification-specific antibody. The limited teachings and shortcomings of the Little and Pidgeon these references were extensively discussed and distinguished by Applicants previously (both in the first in-person interview and in previous Responses), and the Examiner has previously acknowledged that they do not, alone or in combination, render the claimed subject matter obvious. Stoughton, entitled "Methods for Identifying Pathways for Drug Action," fails for the same reasons as Little and Pidgeon.

Finally, the non-obviousness of the present invention over the prior art is irrefutably evidenced by references like Mann, Marcus, Quadroni, Conrads, and others (*see* Refs. CG, CF, CZ and DA-DD (discussed at length above)), all of which teach away from the invention and evidence the failed prior art attempts. The novelty and non-obviousness of Applicants' methods is further evidenced by the surprising results, long-felt but unsolved need, and commercial success associated with the invention, as discussed during the first and second in-person interviews by Dr. John Rush and Applicants' attorney.

Again, the subject matter of claims 3, 6-9, 11, 24-28, 30-39, and 49-52 (as well as all other pending claims) is non-obvious and patentable over Wirth, Little, Pidgeon, and Stoughton (and all other cited references), whether taken alone or together, and the rejection of these claims should be withdrawn.

DOUBLE-PATENTING (STATUTORY) REJECTIONS

The Examiner has again provisionally rejected claims 1-29 under 35 U.S.C. §101 for "statutory" double patenting, as allegedly claiming the same invention as that of claims 1-29 of co-pending application USSN 10/175,486 (Rush *et al.* -- also owned BY CELL SIGNALING TECHNOLOGY, INC., the assignee of the present application).

Since the rejection is *provisional*, Applicants respectfully renew their request that this rejection be held in abeyance until such time as the present application or cited co-pending application issues as a patent, at which time Applicants will cancel or amend any identical claims in the remaining application.

APPLICANTS: Rush *et al.*
U.S.S.N.: 10/777,893

Conclusion

For the reasons set forth above, the outstanding new enablement, novelty, and obviousness rejections of the claims are improper and unsustainable, and should be withdrawn. The present claims are patentable and are in condition for immediate allowance. Withdrawal of the outstanding rejections is respectfully requested, and prompt allowance and issuance of the claims is earnestly solicited.

Similar issuance and allowance of the related parent case (USSN 10/175,486)(Atty. Docket No. CST-201) is also earnestly solicited, as the issues raised in the remaining rejections in that case are the same as those discussed in this paper.

If there are any questions regarding these amendments and remarks, the Examiner and her SPE are requested to call the undersigned attorney at the telephone number provided.

Respectfully submitted,

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(978) 867-2311

Date: August 24, 2006

catalog & technical reference | 2000–2001

Cell Signaling TECHNOLOGY™

[a new company from New England Biolabs]

1

GENERAL PROTEIN MODIFICATION ANTIBODIES

N = 15

Comparison of Activity Versus Phosphorylation Site

Phospho-Akt Substrate Antibody

Phospho-Histone H3 (Ser10) Antibody

Phospho-Tyrosine Monoclonal Antibody (PY20)

Phospho-Tyrosine Monoclonal Antibody (PY99)

Phospho-Threonine Proline Monoclonal Antibody (TPP-Pro-R1)

Phospho-Akt Substrate Antibody

Phospho-14-3-3 Binding Motif Antibody

Phospho-PKA Substrate Antibody

Phospho-(Ser/Thr) Pro Antibody

Nitro-Tyrosine Polyclonal Antibody

Acetylated-Lysine Antibody

Acetylated-Lysine Monoclonal Antibody (Ac-K-103)

Histone H3

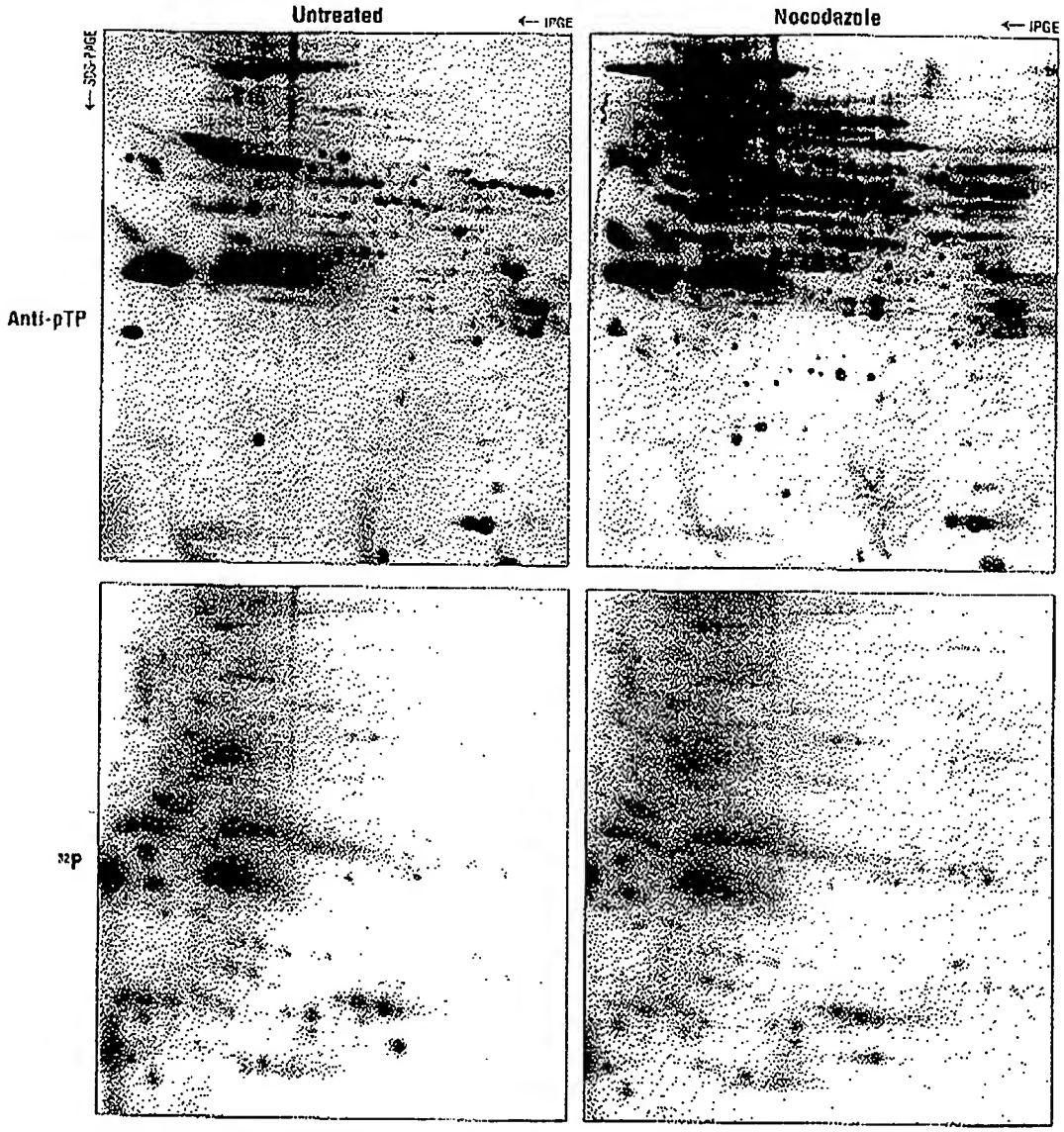
Acetyl-Histone H3 (Lys9) Antibody

Phospho-Histone H3 (Ser10) Antibody

Acetyl- and Phospho-Histone H3 (Lys9/Ser10) Antibody

Histone H3 Antibody

Comparison of Antibody versus ^{32}P-based Phosphoprotein Detection



Antibodies to phospho-threonine-proline are far more sensitive to mitosis-specific protein phosphorylation than is biosynthetic labeling with ^{32}P. Jurkat cells were treated with nocodazole (1 µg/ml) for 12 hours. Aliquots of these cells were simultaneously labeled with [^{32}P]orthophosphate (0.5 mCi/ml). Lysates were separated by 2D gel electrophoresis. Phosphoproteins were revealed in the top two panels by Western analysis using P-Thr-Pro-101 (CST #9391) and in the bottom two panels by [^{32}P]autoradiography.

1

General Protein Modification Antibodies

Phospho-Threonine Antibody (P-Thr-Polyclonal)

Background: Protein kinases are among the most abundant eukaryotic regulatory proteins; over one thousand separate kinase genes are encoded in mammalian genomes (1). In spite of the importance of kinases in eukaryotic biology, relatively few of their physiological targets are known. The Phospho-Threonine Antibody (P-Thr-Polyclonal) provides a powerful new tool for discovering targets of Ser/Thr kinases, for monitoring and characterizing *in vitro* threonine phosphorylation reactions, as well as for high throughput Ser/Thr kinase drug discovery.

Description: P-Thr-Polyclonal, produced using a new patented technology, binds threonine-phosphorylated sites in a manner largely independent of the surrounding amino acid sequence. It recognizes a wide range of threonine-phosphorylated peptides in ELISAs and a large number of threonine-phosphorylated proteins in 2D analyses.

Source: Polyclonal antibodies are produced by immunizing rabbits with synthetic phospho-Thr-containing peptides conjugated to KLH.

Purification: Antibodies are sequentially purified by Protein A and phospho-peptide affinity chromatography.

Specificity: P-Thr-Polyclonal is specific for peptides/proteins containing phospho-Thr and shows no cross-reactivity with corresponding nonphosphorylated sequences. P-Thr-Polyclonal does not cross-react with sequences containing either phospho-Tyr or phospho-Ser.

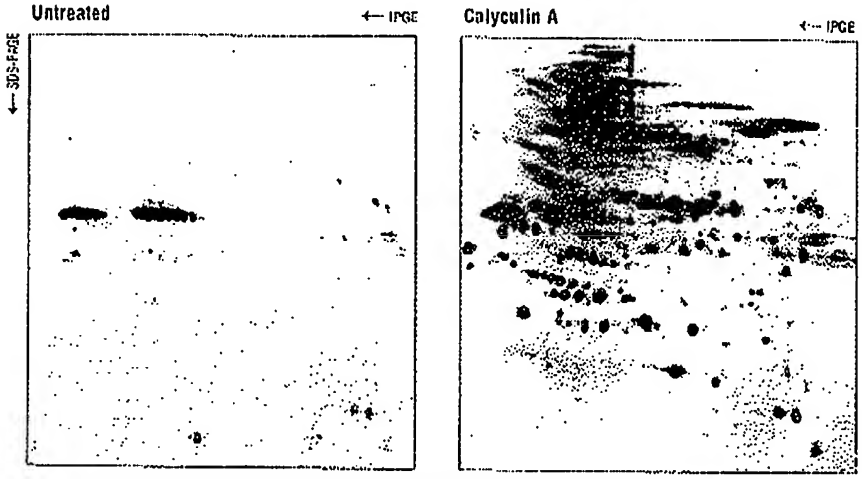
Species Cross-reactivity: It is expected that this antibody will react with threonine-phosphorylated peptides/proteins regardless of species of origin.

Applications: ELISA, Western blotting and immunoprecipitation

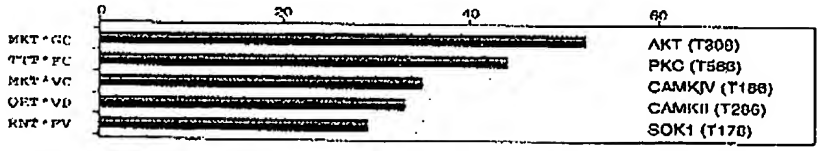
References:

(1) Hunter, T. (2000) *Cell* 100, 113–127.

#9381	100 µl	$250
(10 Western mini-blots)		



Phospho-Threonine Antibody (P-Thr-Polyclonal): Western analysis of whole cell lysates of Jurkat cells untreated and treated with 0.1 µM calyculin A for 45 minutes prior to lysis. Proteins were separated by 2D electrophoresis prior to blotting.



Phospho-Threonine Antibody ELISAs: Signal to noise ratio of phospho- vs. non-phospho-peptides. (T denotes phosphorylated threonine.)*

Phospho-Tyrosine Monoclonal Antibody (P-Tyr-100)

#9411 ~~300µl~~ $195
(40 Western min-blots)

Background: Since the seminal observations that protein tyrosine kinases (PTKs) play critical roles in oncogenic transformation (1,2), it has become clear that both PTKs and phospho-tyrosine phosphatases control a wide range of critical biological processes (3,4). Antibodies specific for phospho-Tyr (5,6) have been invaluable reagents in these studies. The Phospho-Tyrosine Monoclonal Antibodies developed by Cell Signaling Technology (P-Tyr-100 and P-Tyr-102) provide exceptionally sensitive new tools for studying Tyr phosphorylation and monitoring Tyr kinase activity in high throughput drug discovery.

Description: P-Tyr-100 is a high affinity anti-phospho-Tyr monoclonal antibody. ELISAs using a wide variety of phospho-peptides indicate (1) that P-Tyr-100 binds phospho-Tyr in a manner largely independent of the surrounding amino acid sequence, and (2) that P-Tyr-100 binds to a larger number of phospho-Tyr containing peptides than other anti-phospho-Tyr monoclonal antibodies including 4G10 and PY20. 2D-Western blot analyses of pervanadate-treated Jurkat lysates indicates that P-Tyr-100 interacts with a broader range of Tyr-phosphorylated proteins than does 4G10.

Source: P-Tyr-100 (IgG1) is a monoclonal antibody that was derived from a mouse immunized with phospho-Tyr peptides coupled to KLH.

Purification: P-Tyr-100 antibody is supplied as mouse ascites fluid.

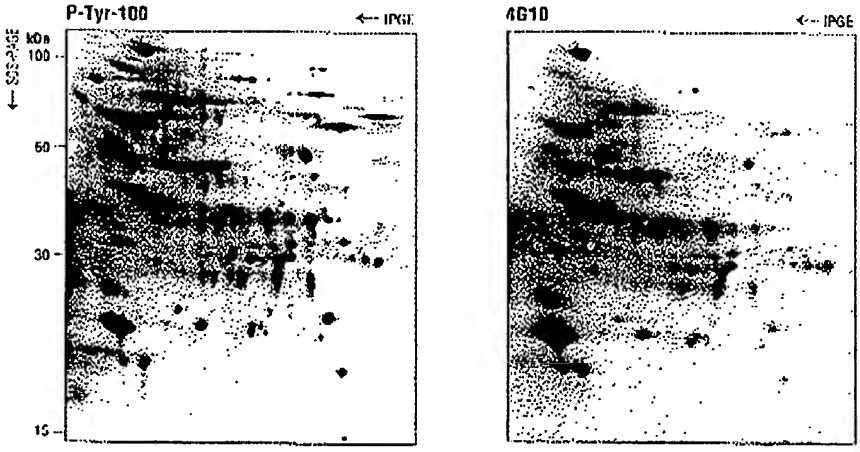
Specificity: P-Tyr-100 is highly specific for phospho-Tyr in peptides/proteins, shows no cross-reactivity with the corresponding nonphosphorylated peptides, and does not cross-react with peptides containing phospho-Ser or phospho-Thr instead of phospho-Tyr.

Species Cross-reactivity: P-Tyr-100 will react with peptides/proteins containing phospho-Tyr from all species.

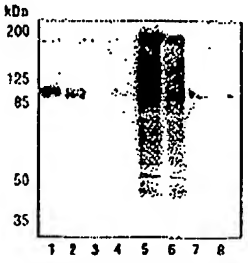
Applications: ELISA, Western blotting, immunoprecipitation

References:

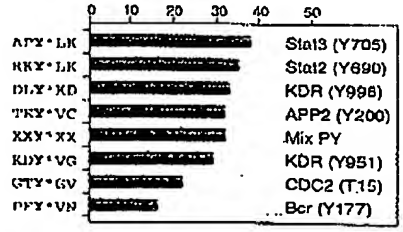
(1) Varmus, H. and Bishop, J.M. (1986) *Cancer Surv.* 5, 153–158.
(2) Hunter, T. and Cooper, J.A. (1985) *Annu. Rev. Biochem.* 54, 897–930.
(3) Fischer, E.H. (1999) *Adv. Enzyme Regul.* 39, 359–369.
(4) Hunter, T. (1997) *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 353, 583–605.
(5) Ward, S.G. et al. (1992) *J. Biol. Chem.* 267, 23862–23869.
(6) Glenney, J.R. (1988) *J. Immunol. Methods* 109, 277–285.



Comparison of P-Tyr-100 and 4G10 Phospho-Tyrosine Antibodies: Western analysis of whole cell lysates of Jurkat cells treated with 1 mM pervanadate for 30 minutes prior to lysis. Proteins were separated by 2D electrophoresis prior to blotting.



Tyrosine phosphorylation in rat hippocampal dentate gyrus ischemia. Control (Lanes 1,2); 15 minute transient cerebral ischemia (Lanes 3,4); reperfusion for 4 hours (Lanes 5,6); and 24 hours (Lanes 7,8). Western analysis using P-Tyr-100.



Phospho-Tyrosine Antibody (P-Tyr-100) ELISAs: signal to noise ratio of phospho- vs. non-phospho-peptides. (Y denotes phosphorylated tyrosine.)*

Phospho-Tyrosine Monoclonal Antibody (P-Tyr-102)

Description: P-Tyr-102 is a high affinity, IgM monoclonal antibody. ELISAs using a wide variety of phospho-peptides indicate that P-Tyr-102 binds phospho-Tyr in a manner largely independent of the surrounding amino acid sequence. 2D-Western blot analyses indicate that P-Tyr-102 binds strongly to a broader range of tyrosine-phosphorylated proteins than PY-20. Its strength of binding suggests that P-Tyr-102 may be useful in applications that require especially high avidities. Its fine specificity seems to vary slightly from that of P-Tyr-100. Additionally, because it is an IgM, it may easily be used in conjunction with antibodies of different isotypes for multiplex analyses.

Source: P-Tyr-102 (IgM) is a mAb that was derived from a mouse immunized with synthetic phospho-Tyr peptides coupled to KLH.

Purification: P-Tyr-102 antibody is supplied as mouse ascites fluid.

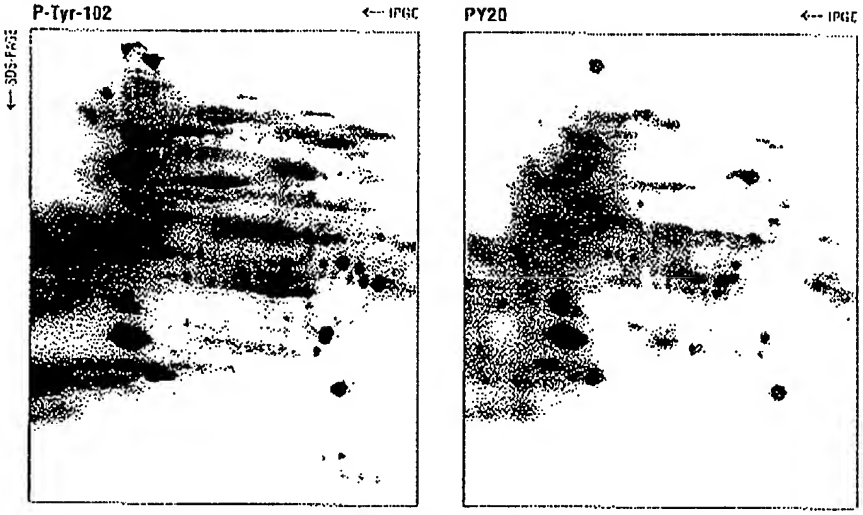
Specificity: P-Tyr-102 is highly specific for phospho-Tyr in peptides /proteins, shows no cross-reactivity with the corresponding nonphosphorylated peptides, and does not react with peptides containing phospho-Ser or phospho-Thr instead of phospho-Tyr.

Species Cross-reactivity: P-Tyr-102 will react with peptides/proteins containing phospho-Tyr from all species.

Applications: ELISA, Western blotting

#9416 (40 Western mini-blots)	100 µl	$195

Western analysis of extracts from sodium vanadate treated (3 mM for 0.5 hour) NIH/3T3 cells using Phospho-Tyrosine Monoclonal Antibody (P-Tyr-102)



Comparison of P-Tyr-100 and PY20 Phospho-Tyrosine Antibodies: Western analysis of whole cell lysates of Jurkat cells treated with 1 mM pervanadate for 30 minutes prior to lysis. Proteins were separated by 2D electrophoresis prior to blotting.

Phospho-Threonine-Proline Monoclonal Antibody (P-Thr-Pro-101)

#9391S	100 µl	$195
(50 Western mini-blots)		
#9391L	300 µl	$475
(150 Western mini-blots)		

Background: Proline-directed protein kinases, including MAPKs, Cdks, and GSKs, can phosphorylate Ser/Thr residues followed by prolines (1). Pro-directed phosphorylation plays a central role in many critical biological processes including transcriptional regulation (2), checkpoint control (3), apoptosis (4), carcinogenesis (5), and neurological degeneration (6) yet few of the physiological substrates of these kinases are known. The phospho-Thr-Pro Monoclonal Antibody (P-Thr-Pro-101) provides a powerful new tool for investigating the cellular role of proline-directed phosphorylation, as well as monitoring and characterizing the *in vitro* activity of Pro-directed protein kinases and phosphatases.

Description: The P-Thr-Pro-101 Antibody binds the phospho-Thr-Pro motif in a manner largely independent of the surrounding amino acid sequence in ELISAs. 2D Western analyses indicate that P-Thr-Pro-101 specifically detects a broad range phosphorylated proteins.

Source: The monoclonal antibody P-Thr-Pro-101 (IgM) was derived from a mouse immunized with synthetic phospho-Thr-Pro peptides coupled to KLH.

Purification: P-Thr-Pro-101 antibody is purified from ascites fluid by Protein A chromatography.

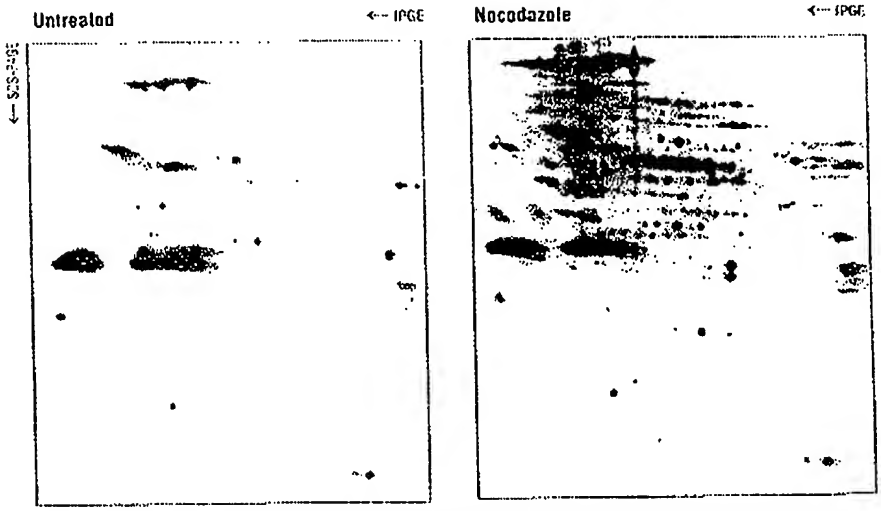
Specificity: P-Thr-Pro-101 Antibody is highly specific for peptides containing phospho-Thr-Pro. It reacts neither with nonphosphorylated peptides with the same sequence nor with threonine-phosphorylated peptides lacking downstream prolines. P-Thr-Pro-101 Antibody does not react with phospho-Tyr but does cross-react with 1 of 30 phospho-Ser peptides that have been tested.

Species Cross-reactivity: It is expected that this antibody will react with peptides/proteins containing phospho-Thr-Pro from all species.

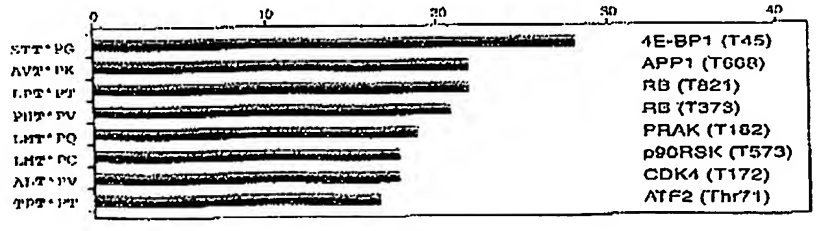
Applications: ELISA, Western blotting

References:

(1) Pearson, R.B. and Kemp, B.E. (1991) *Meth. Enzymol.* 200, 62–81.
(2) Seger, R. and Krebs, E.G. (1995) *FASEB J.* 9, 726–735.
(3) Nurse, P. (2000) *Cell* 100, 71–78.
(4) Cross, T.G. et al. (2000) *Exp. Cell Res.* 256, 34–41.
(5) Yang, C.C. et al. (1998) *J. Prot. Chem.* 17, 329–335.
(6) Reynolds, C.H. et al. (2000) *J. Neurochem.* 74, 1587–1595.



Phospho-Threonine-Proline Monoclonal Antibody (P-Thr-Pro-101): Western analysis of whole cell lysates of Jurkat cells untreated and treated with 1 µg/ml nocadazole for 12 hours prior to lysis. Proteins were separated by 2D electrophoresis prior to blotting.



Phospho-Threonine-Proline Monoclonal Antibody ELISAs: Signal to noise ratio of phospho- vs. non-phospho peptides. (T denotes phosphorylated threonine.)*

1 General Protein Modification Antibodies

Phospho-Akt Substrate Antibody

Background: Akt plays a central role in mediating critical cellular responses including cell growth and survival (1), angiogenesis (2), and transcriptional regulation (3). While a number of Akt substrates including GSK3, Bad, and Caspase 9 are known (4–6), many important substrates await discovery (7). Akt phosphorylates substrates only at Ser/Thr in a conserved motif characterized by Arg at positions –5 and –3 (8). The Phospho-Akt Substrate Antibody recognizes phosphorylated Akt substrates, providing a powerful new tool for the discovery and characterization of potential Akt target proteins.

Description: Phospho-Akt Substrate Antibody, produced using a new patented technology, binds preferentially to peptides/proteins containing phospho-Thr/Ser preceded by Lys/Arg at positions –5 and –3 in a manner largely independent of the surrounding amino acid sequence. It recognizes a wide range of phosphorylated Akt substrate peptides in ELISAs and a large number of presumptive Akt substrates in 2D analyses.

Source: Polyclonal rabbit antibodies

Purification: Antibodies are sequentially purified on Protein A and peptide affinity chromatography.

Specificity: This antibody preferentially binds peptides/proteins which contain phospho-Thr/Ser preceded by Arg/Lys at positions –5 and –3. Some cross-reactivity is observed for peptides that contain phospho-Thr/Ser preceded by Arg/Lys at positions –3 and –2. No cross-reactivity is observed with corresponding nonphosphorylated sequences or with other phospho-Thr/Ser containing motifs.

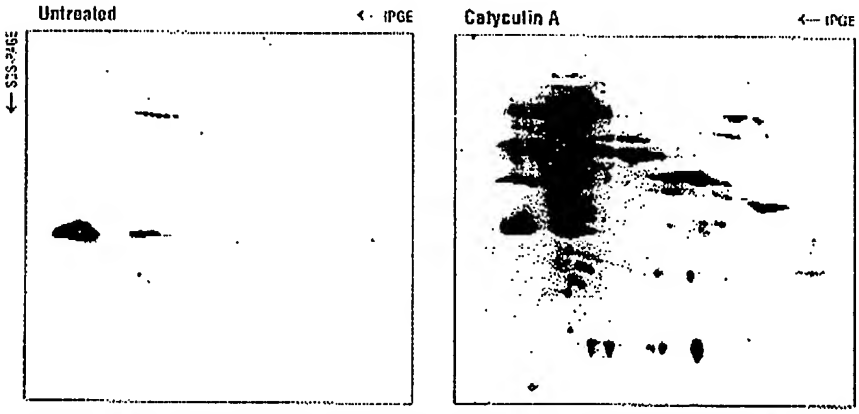
Species Cross-reactivity: It is expected that this antibody will react with the specified phosphorylated motif from all species.

Applications: ELISA, Western blotting

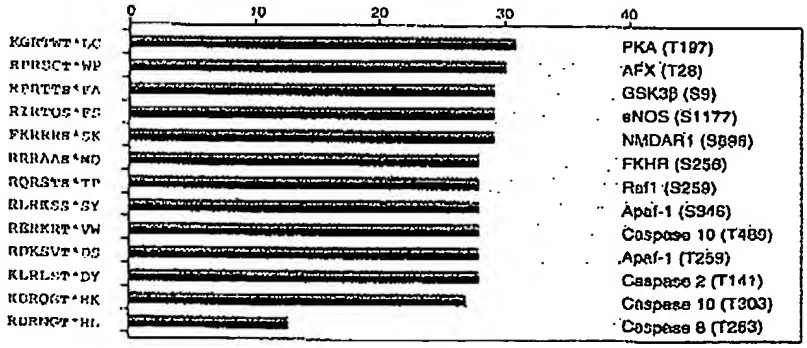
References:

(1) Marte, B.M. and Downward, J. (1997) *Trends Biochem. Sci.* 22, 355–358.
(2) Jiang, B.H. et al. (2000) *Proc. Natl. Acad. Sci. USA* 97, 1749–1753.
(3) Scheid, M.P. and Woodgett, J.R. (2000) *Curr. Biol.* 10, R191–194.
(4) Pap, M. et al. (1998) *J. Biol. Chem.* 273, 19929–19932.
(5) Datta, S.R. et al. (1997) *Cell* 91, 231–241.
(6) Cardone, M.H. et al. (1998) *Science* 282, 1318–1321.
(7) Coffer, P.J. et al. (1998) *Biochem. J.* 335, 1–13.
(8) Alessi, D.R. et al. (1996) *FEBS Lett.* 399, 333–338.

#9611S	100 µl	$250
(10 Western mini-blots)		
#9611L	300 µl	$600
(30 Western mini-blots)		



Phospho Akt Substrate Antibody: Western analysis of whole cell lysates of Jurkat cells untreated and treated with 0.1 mM calyculin A for 30 minutes prior to lysis. Proteins were separated by 2D electrophoresis prior to blotting.



Phospho-Akt Substrate Antibody ELISAs: Signal to noise ratio of phospho vs. non-phospho peptides corresponding to potential Akt substrate sites. (T and S* denote phosphorylated threonine and serine.)*

1

General Protein Modification Antibodies

Phospho-14-3-3 Binding Motif Antibody

#9601S (10 Western mini-blots)	100 µl	$250
#9601L (30 Western mini-blots)	300 µl	$600

Background: The 14-3-3 proteins are a highly conserved family of proteins involved in the regulation of cell survival, apoptosis, proliferation and checkpoint control (1–5). Biological regulation by 14-3-3 is mediated through phosphorylation-dependent protein-protein interactions (6). Two different phospho-Ser-containing motifs are found within nearly all known 14-3-3 binding proteins (7). Motif 1 (Arg/Lys and Ser at positions –3 and –2, phospho-Ser at position 0, and Pro at position +2) is found in critical regulatory proteins including Bad, cdc25c, FKHRL1, PKC and c-Raf (5,7). This antibody provides a powerful new tool for the discovery and characterization of potential 14-3-3 binding proteins and for high throughput drug discovery.

Description: Phospho-14-3-3 Binding Motif Antibody, produced using a new patented technology, binds peptides/proteins containing phospho-Ser surrounded by Pro at the +2 position and Arg/Lys at the –3 position. Binding is phospho-specific and largely independent of the surrounding amino acid sequence. It recognizes a wide range of peptides containing phosphorylated 14-3-3 binding motifs in ELISAs and a large number of presumptive 14-3-3 binding proteins in 2D analyses.

Source: Polyclonal rabbit antibodies

Purification: Antibodies are sequentially purified on Protein A and peptide affinity chromatography.

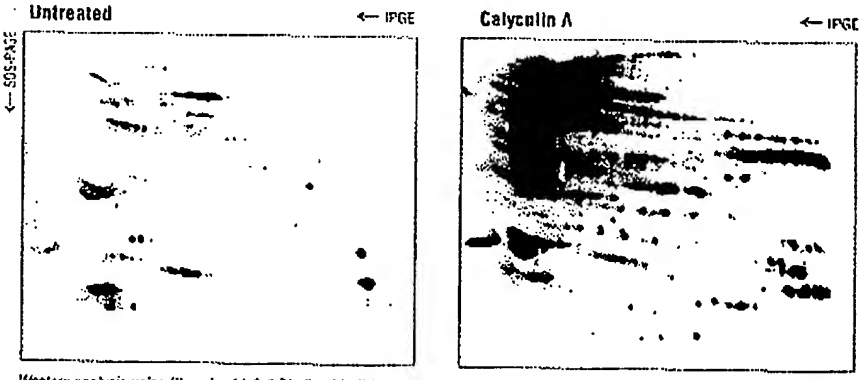
Specificity: This antibody is highly specific for peptides/proteins containing phospho-Ser surrounded by Pro at the +2 position and Arg or Lys at the –3 position. It weakly cross-reacts with analogous sequences containing phospho-Thr instead of phospho-Ser in this motif and with sequences containing phospho-Ser surrounded by Phe at the +1 position and Arg/Lys at the –3 position. No cross-reactivity is observed with corresponding nonphosphorylated sequences or with other phospho-Thr/Ser/Tyr containing motifs.

Species Cross-reactivity: It is expected that this antibody will react with the specified phosphorylated motif from all species.

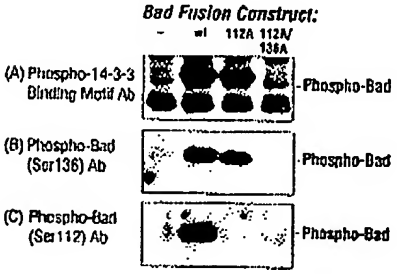
Applications: Western blotting, ELISA

References:

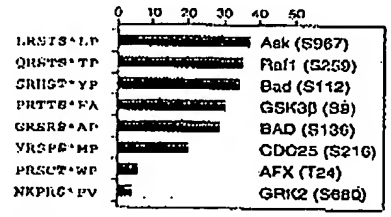
(1) Aitken, A. (1995) *Trends Biochem. Sci.* 20, 95–97.
(2) Zha, J. et al. (1996) *Cell* 87, 619–628.
(3) Piwnica-Worms, H. (1999) *Nature* 401, 535–537.
(4) Tzivion, G. et al. (1998) *Nature* 394, 88–92.
(5) Xing, H. (2000) *EMBO J.* 19, 349–358.
(6) Muslin, A.J. (1996) *Cell* 84, 889–897.
(7) Yaffe, M.B. et al. (1997) *Cell* 91, 961–971.



Western analysis using Phospho-14-3-3 Binding Motif Antibody. Jurkat cells were treated with 0.1 mM calyculin A for 30 minutes, lysed, and separated by 2D electrophoresis prior to blotting.



Western analysis of Bad or Bad mutant fusion protein transfected 293 cells using (A) Phospho-14-3-3 Binding Motif Antibody, (B) Phospho-Bad (Ser136) Antibody, and (C) Phospho-Bad (Ser112) Antibody.



Phospho-14-3-3 Binding Motif Antibody ELISAs: signal/noise ratio of phospho/non-phospho 14-3-3 binding motif peptides. T and S* denote phosphorylated threonine and serine.)*

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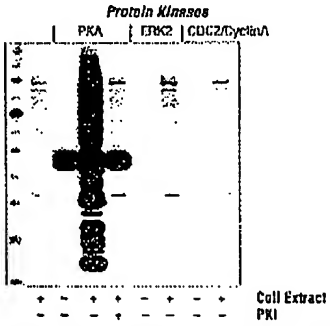
Phospho-PKA Substrate Antibody

Background: A class of kinases that regulate an array of physiological processes and include cAMP-dependent protein kinase (PKA), cGMP-dependent protein kinase, protein kinase C, Akt, and RSK share a substrate specificity characterized by an Arg at position –3 relative to the phosphorylated Ser or Thr (1,2). These are referred to as Arg-directed kinases or AGC-family kinases. The Phospho-PKA Substrate Antibody provides a powerful new tool for investigating the regulation of phosphorylation by PKA and other Arg-directed kinases, as well as for high throughput kinase drug discovery.

Description: Phospho-PKA Substrate Antibody detects peptides/proteins containing phospho-Thr with Arg at the –3 position. It is a useful tool in identifying new substrates of AGC-family kinases, including PKA and PKC.



Western analysis of A431 cell extracts in vitro phosphorylated by protein kinase A, plus or minus PKA inhibitor (PKI), ERK2 and CDC2/cyclinA using phospho-PKA Substrate Antibody.

Source: Polyclonal rabbit antibodies

Purification: Antibodies are sequentially purified on Protein A and peptide affinity chromatography.

Specificity: This antibody is specific for peptides contain phospho-Thr with Arg at the –3 position. It also recognizes some peptides containing phospho-Ser with Arg at the –2 and –3 position. It does not recognize the nonphosphorylated PKA substrate motif.

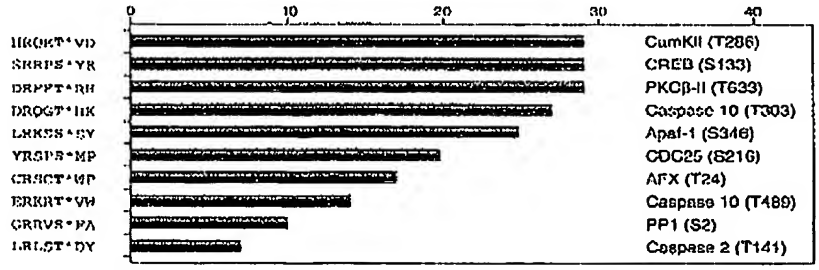
Species Cross-reactivity: This antibody should react with peptides/proteins containing the specified phosphorylated motif from all species.

Applications: Western blotting, ELISA, immunoprecipitation

References:

(1) Mordminy, M. (1997) *Annu. Rev. Biochem.* 66, 807–822.
(2) Pearson, R.B. and Kemp, B.E. (1991) *Methods Enzymol.* 200, 62–81.

Western analysis of calyculin A-treated A431 cells using phospho-PKA Substrate Antibody.



Phospho-PKA Substrate Antibody ELISAs: Signal to noise ratio of phospho vs. non-phospho peptides containing arginine or lysine at the –3 position. (T and S* denote phosphorylated threonine and serine.)*

#9621S (10 Western mini-blots)	100 µl	$250
#9621L (30 Western mini-blots)	300 µl	$600

Phospho-(Ser/Thr) Phe Antibody

#9631S (10 Western mini-blots)	100 µl	$250
#9631L (30 Western mini-blots)	300 µl	$600

Background: Many critical protein kinases can be regulated by phosphorylation at a specific serine or threonine surrounded by phenylalanine or tyrosine. For example, Akt, an important kinase that regulates cell survival, is activated by phosphorylation at Ser473, a site surrounded by phenylalanine and tyrosine (1). RSK1, p70S6K, and certain PKC isoforms also contain a similar consensus phosphorylation site. Phosphorylation of these sites is required for kinase activity (2,3). The Phospho-(Ser/Thr) Phe Antibody is a powerful new tool for discovery of new proteins containing this important regulatory motif.

Description: Phospho-Serine/Threonine Phenylalanine Antibody detects phosphorylated serine or threonine that requires tyrosine, tryptophan, or phenylalanine at the -1 position or phenylalanine at the +1 position.

Source: Polyclonal rabbit antibodies.

Purification: Antibody is purified by protein A and peptide affinity chromatography.

Specificity: The antibody is highly specific to phosphorylated [F/Y/W][T/S] or [S/T]F containing peptides and proteins, and does not recognize nonphosphorylated [F/Y/W][T/S] or [S/T]F motifs or other phospho-serine/threonine containing proteins and peptides.

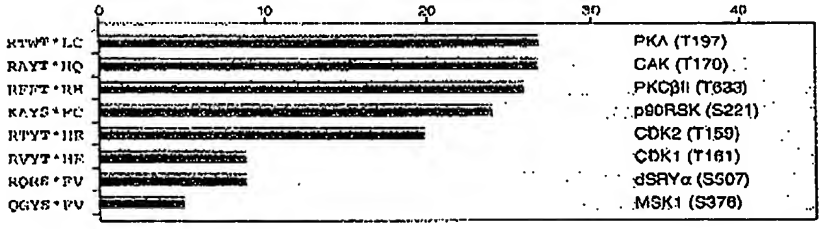
Species Cross-reactivity: This antibody should react with peptides/proteins containing the phosphorylated motif from all species.

Applications: ELISA, Western blotting, immunoprecipitation

References:

(1) Alessi, D.R. et al. (1996) *EMBO J.* 15, 6541–6551.
(2) Dalby, K.N. et al. (1998) *J. Biol. Chem.* 273, 1496–1505.
(3) Keranen, L.M. et al. (1995) *Curr Biol.* 5, 1394–1403.

Western analysis of calyculin A treated A431 cells using Phospho-Serine/Threonine Phenylalanine Antibody.



Phospho-Serine/Threonine Phenylalanine Antibody ELISAs: signal to noise ratio of phospho vs. non-phospho peptides in which the phosphorylation site is adjacent to phenylalanine, tyrosine, or tryptophan. (T and S* denote phosphorylated threonine and serine.)*

1 General Protein Modification Antibodies

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Nitro-Tyrosine Polyclonal Antibody

Background: Nitric oxide (NO) is implicated in carcinogenesis (1), chronic infection, inflammation (2), and neurodegeneration (3). High levels of both superoxide and nitric oxide in these tissues interact to form peroxynitrite, a potent oxidant that can modify Tyr residues in proteins to form 3-nitrotyrosine (4). Tyrosine nitration of mitochondrial manganese superoxide dismutase results in loss of enzymatic activity (4). The nitration of p53 at Tyr residues abolishes its capacity for binding to its DNA consensus sequence (5).

Description: Nitro-Tyrosine Antibody detects proteins/peptides with nitro-Tyr independent of the surrounding amino acid sequences. It is a valuable tool for identifying new nitrated proteins as well as for diagnosis of protein nitration and measuring levels of nitrated proteins in tissues and samples.

Source: Polyclonal antibodies are produced by immunizing rabbits with a synthetic nitro-Tyr-containing peptide coupled to KLH.

Purification: Antibodies are purified sequentially by protein A and peptide affinity chromatography.

Specificity: Nitrotyrosine Antibody is highly specific for nitrated Tyr. It does not recognize native tyrosine residues or phospho-Tyr.

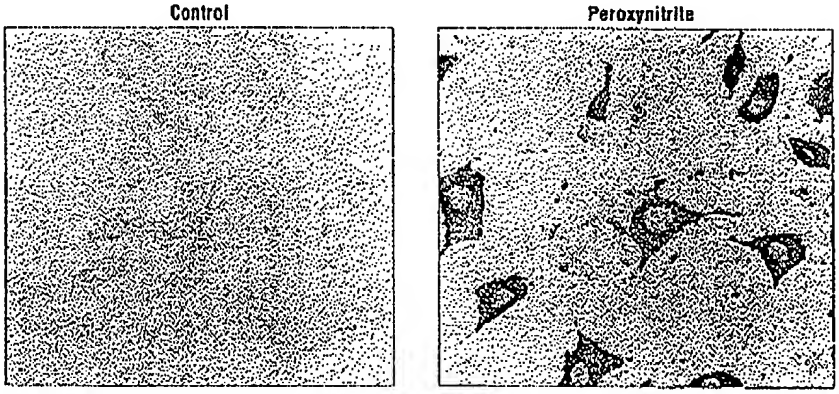
Species Cross-reactivity: It is expected that Nitro-Tyrosine Antibody will react with peptides/proteins containing nitro-Tyr from all species.

Applications: Western blotting, ELISA, immunocytochemistry

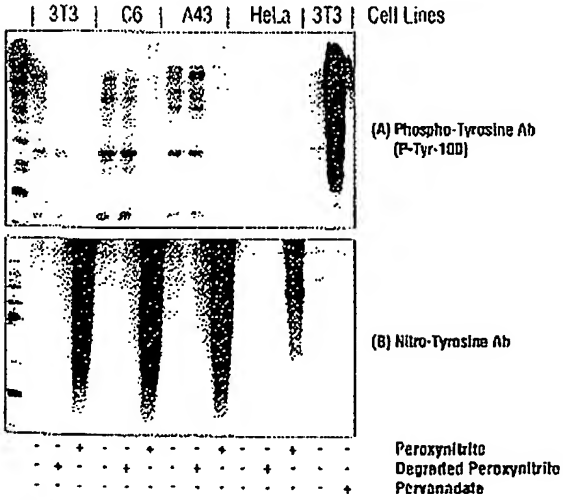
References:

(1) Bentz, B.G. et al. (2000) *Head Neck* 22, 64–70.
(2) Jaiswal, M. et al. (2000) *Cancer Res.* 60, 184–190.
(3) Olivenza, R. et al. (2000) *J. Neurochem.* 74, 785–791.
(4) MacMillan-Crow, L.A. et al. (1996) *Proc. Natl. Acad. Sci. USA* 93, 11853–11858.
(5) Chazotte-Aubert, L. et al. (2000) *Biochem. Biophys. Res. Commun.* 267, 609–613.

#9691S	100 µl	$250
(10 Western mini-blots)		
#9691L	300 µl	$600
(30 Western mini-blots)		



Immunocytochemistry staining of 3T3 cells treated with degraded peroxynitrite (control) or peroxynitrite using Nitrotyrosine Antibody (brown).



Western analysis of whole cell lysates of different cells untreated, treated with peroxynitrite, degraded peroxynitrite, or pervanadate using (A) Phospho-Tyrosine Antibody, (B) Nitrotyrosine Antibody.

1

General Protein Modification Antibodies

Acetylated-Lysine Antibody

#9441S (10 Western mini-blots)	100 µl	$250
#9441L (30 Western mini-blots)	300 µl	$600

Background: Protein acetylation on lysine, like phosphorylation of serine, threonine or tyrosine is an important reversible modification controlling protein activity. The conserved amino-terminal domains of the four core histones (H2A, H2B, H3 and H4) contain lysines that are acetylated by histone acetyltransferases (HATs) and deacetylated by histone deacetylases (HDACs) (1). Acetylation of histones and transcription factors is well known to regulate chromatin structure and gene activity (2–4).

To study and discover new sites of protein acetylation, Cell Signaling Technology has developed a patented method to generate polyclonal antibodies that recognize acetylated-lysine in a manner largely independent of the surrounding amino acid sequence. Western blot analysis of extracts from Trichostatin A (TSA; a deacetylase inhibitor) treated cells shows that the Acetylated-Lysine Antibody reacts with a variety of acetylated proteins.

Description: Acetylated-Lysine Antibody detects proteins containing sites of posttranslational acetylation on the ε-amino groups of lysine residues. It recognizes acetylated-lysine in many contexts. It has been demonstrated to recognize acetylated histones, p53, CBP, PCAF, and chemically acetylated BSA.

Source: Polyclonal antibodies are produced by immunizing rabbits with a synthetic acetylated-lysine peptide (KLH coupled).

Purification: Antibodies are purified by protein A and peptide affinity chromatography.

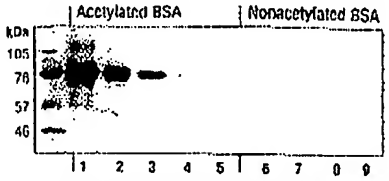
Sensitivity and Specificity: The Acetylated-Lysine Antibody is acetylation specific. It specifically reacts with as little as 0.04 ng of chemically acetylated BSA without cross-reacting with up to 25 µg of nonacetylated BSA.

Species Cross-reactivity: It is expected that this antibody will react with acetylated peptides/proteins from all species.

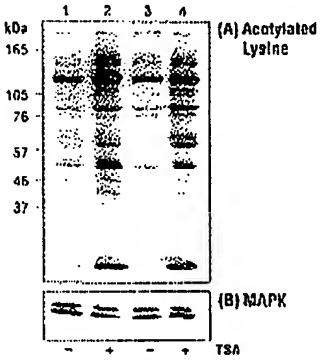
Applications: Western blotting, immunoprecipitation, ELISA

Selected Application References:
Martinez-Balbas, M.A. et al. (2000) Regulation of E2F1 activity by acetylation. *EMBO J.* 19(4), 662–671.

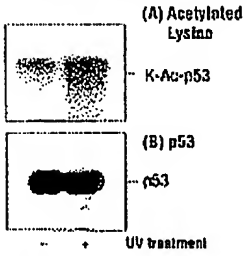
References:
(1) Hassig, C.A. and Schreiber, S.L. (1997) *Curr. Opin. Chem. Biol.* 1(3), 300–308.
(2) Allfrey, V.G. et al. (1964) *Proc. Natl. Acad. Sci. USA* 51, 786–794.
(3) Lin, L. et al. (1999) *Mol. Cell. Biol.* 19(2), 1202–1209.
(4) Boyes, J. et al. (1998) *Nature* 396, 594–498.



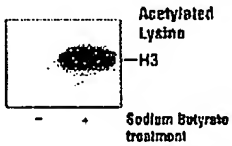
Specificity and sensitivity of Acetylated-Lysine Antibody assayed on acetylated BSA (4, 1, 0.2, 0.04 or 0.008 ng; lanes 1–5) or nonacetylated BSA (25,000, 5,000, 1,000 or 200 ng; lanes 6–9).



Western analysis of extracts from TSA-treated COS Cells grown in 10% FBS (lanes 1–2) or serum starved for 18 hours (lanes 3–4) using Acetylated-Lysine Antibody (A) and MAPK Antibody (B).



Increase in p53 acetylation of UV-treated 293 cells immunoprecipitated with p53 Antibody, then detected by Western blotting using Acetylated-Lysine (A) and p53 Antibodies (B).



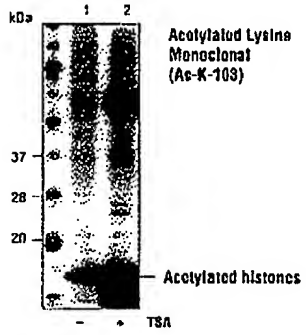
Increase in Histone acetylation of 3T3 cells treated with 5 mM Sodium Butyrate for 24 hours detected by Western blotting using Acetylated-Lysine Antibody.

Acetylated-Lysine Monoclonal Antibody (Ac-K-103)

Background: Protein acetylation on lysine, like phosphorylation of serine, threonine or tyrosine, is an important reversible modification controlling protein activity. The conserved amino-terminal domains of the four core histones (H2A, H2B, H3 and H4) contain lysines that are acetylated by histone acetyltransferases (HATs) and deacetylated by histone deacetylases (HDACs) (1). Acetylation of histones and transcription factors is well known to regulate chromatin structure and gene activity (2–4).

To study and discover new sites of protein acetylation, Cell Signaling Technology has developed a patented technology to generate antibodies that recognize acetylated lysine in a fashion largely independent of the surrounding amino acid sequence. Western analysis using extracts from Trichostatin A (TSA; a deacetylase inhibitor) treated cells shows that the Acetylated-Lysine Antibody reacts with a wide variety of acetylated proteins.

Description: Ac-K-103 detects proteins containing sites of posttranslational acetylation on the ε-amino groups of lysine residues. As a mouse monoclonal antibody, Ac-K-103 complements our Acetylated-Lysine Polyclonal Antibody (catalog #9441) as it shows different and overlapping specificity. Acetylated-Lysine Monoclonal Antibody (Ac-K-103) demonstrates context-independent detection of acetylated lysine. In addition to acetylated histones, which it detects particularly well, the antibody also has been shown to recognize CBP, PCAF, as well as chemically acetylated BSA.



Western analysis of COS cell extracts treated with 0.4 mM TSA for 18 hours using Acetylated-Lysine Monoclonal Antibody (Ac-K-103).

Source: Monoclonal antibody is produced by immunizing mice with a synthetic acetylated-lysine peptide (KLH coupled). Acetylated-Lysine Monoclonal Antibody is a mouse IgG2a isotype.

Purification: The antibody is supplied as mouse ascites fluid.

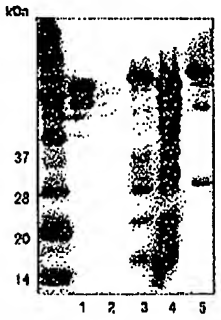
Specificity: The Acetylated-Lysine Antibody is acetylation specific. It specifically reacts with acetylated-lysine without cross-reacting with nonacetylated lysine.

Species Cross-reactivity: It is expected that this antibody will react with acetylated peptides/proteins from all species.

Applications: Western blotting, ELISA

References:

(1) Hassig, C.A. and Schreiber, S.L. (1997) *Curr. Opin. Chem. Biol.* 1(3), 300–308.
(2) Allfrey, V.G. et al. (1964) *Proc. Natl. Acad. Sci. USA* 51, 786–794.
(3) Liu, L. et al. (1999) *Mol. Cell. Biol.* 19, 1202–1209.
(4) Hung, H.L. et al. (1999) *Mol. Cell. Biol.* 19, 3496–3505.



Western analysis of NIH/3T3 cell extracts in vitro acetylated by CPB or PCAF, then detected with Acetylated-Lysine Monoclonal Antibody (Ac-K-103). (Lanes 1 and 5 are PCAF and CBP auto acetylation, lanes 2–4 are acid extracts from 3T3 cells control, acetylated with CBP, acetylated with PCAF, respectively.)

#9681S (40 Western mini-blots)	200 µl	$195
#9681L (120 Western mini-blots)	600 µl	$475

Motif